Clinical Endocrinology (2006) **65**, 423–428 doi: 10.1111/j.1365-2265.2006.02578.x

ORIGINAL ARTICLE

Lower ability to oxidize lipids in adult patients with growth hormone (GH) deficiency: reversal under GH treatment

F. Brandou*†, I. Aloulou*, A. Razimbaud*, C. Fédou*, J. Mercier*† and J. F. Brun*

**Service Central de Physiologie Clinique, Centre d'Exploration et de Réadaptation des Anomalies du Métabolisme musculaire (CERAMM), CHU Montpellier, and †Département de Physiologie des Interactions, EA 701 'Muscles et Pathologies chroniques', Université Montpellier 1, Faculté de Médecine, Montpellier, France*

Summary

Background The aim of the study was to characterize lipid oxidation at exercise in adults with growth hormone deficiency (GHD) and to evaluate the effect of 6 and 12 months of GH replacement therapy on substrate carbohydrate (CHO) and lipid utilization at exercise.

Patients and measurements Twenty-five patients with GHD and 40 matched controls participated in the study. Ten of the 25 GH-deficient patients were treated with recombinant GH for 12 months. Anthropometric measurements and exercise calorimetry were performed before and after treatment. Maximal fat oxidation and the crossover point [that is the percentage of the theoretical maximal power (Wmax th) where CHO become the predominant fuel used for oxidation] were determined.

Results and conclusion The GH-deficient patients exhibited a highly significant shift in the balance of substrate oxidation during exercise, towards a decrease in fat oxidation, and a shift towards lower intensities of the crossover ($52 \pm 5{\cdot}5\%$ *vs.* $72{\cdot}6 \pm 6{\cdot}6\%$ of Wmax th, $P < 0{\cdot}03$) and maximal fat oxidation ($131{\cdot}04 \pm 14$ *vs.* $234{\cdot}4 \pm 30{\cdot}1$ mg/min, $P < 0{\cdot}03$) in the GHD and control groups, respectively. However, GH treatment at 6 and 12 months partially reversed this defect, resulting in an increase (+83%, $P < 0{\cdot}001$) in the maximal ability to oxidize fat during exercise. These findings are consistent with the hypothesis that a lack of GH reduces the ability to oxidize lipids during exercise and that GH treatment restores this muscular metabolic property.

(Received 26 October 2005; returned for revision 3 January 2006; finally revised 7 February 2006; accepted 10 February 2006)

Introduction

Adult growth hormone deficiency (GHD) results in abnormalities in body composition,[1,2] fuel metabolism[3,4] and exercise performance.[5] Many studies have reported changes in body composition in GH-deficient patients and their reversal under GH replacement. In general, GH-deficient patients have been shown consistently to have reduced skeletal muscle, reduced fat free mass (FFM) and increased fat mass.[6–8] Fat is accumulated in a central distribution, mostly in visceral tissue. These defects are associated with impaired insulin sensitivity[9,10] and result in adverse metabolic effects. Replacement therapy with recombinant GH tends to normalize these defects and provides physical and psychological benefits.[11,12] Because of its anabolic and lipolytic actions, GH improves body composition, muscle mass and physical fitness, which are altered in GHD. Indeed, in all reported studies investigating the effects of GH treatment on body composition, an increase in FFM with reductions in fat mass[7,13] has been reported. It is also known that adult patients with GHD have a low basal metabolic rate that is enhanced with GH treatment.[6]

An important mechanism of the body's adaptation to exercise is the availability and selection of energetic substrates used for oxidation during muscular activity. Standardized protocols have recently been proposed to assess this balance between lipids and carbohydrates (CHO) oxidized during exercise with indirect calorimetry.[14–16] With this approach, the balance of substrates at exercise has been studied in different populations (sedentary, children, diabetics, obese, athletes), but its pattern in GHD, as well its alterations under GH treatment, remain unknown.

Therefore, the aim of this study was first to describe the balance of energetic substrates at exercise in GHD (compared to matched controls), and also to evaluate the effect of 6 and 12 months of GH replacement therapy in these patients.

Subjects and methods

Patients

Twenty-five patients with GHD and 40 controls matched for age and body composition participated in the study. Diagnosis of GHD was established according to international consensus recommendations.[17] Anthropometric and morphological characteristics are shown in Table 1. GHD was diagnosed by clinical characteristics and GH response to insulin hypoglycaemia or glucagon propranolol stimulation tests. GH maximal response had to be less than 10 μUI/ml.

To evaluate 6 and 12 months of GH replacement therapy, 10 of the 25 GH-deficient patients (seven women and three men, mean age 43·1 years, mean weight 71·4 kg) were treated with GH

Correspondence: Frédérique Brandou, Institut de Biologie, Bd Henry IV, 34060 Montpellier, France. Tel.: 04 67 60 07 66; Fax: 04 67 60 69 04; E-mail: fredbrandou@yahoo.fr

Table 1. Anthropometric characteristics in GHDA and controls group

	GHD ($n = 25$)	Controls ($n = 40$)	P
Sex ratio (M/F)	11/14	17/23	
Age (years)	36·7 ± 2·6	36·5 ± 1·4	NS
BMI (kg/m^2)	26·6 ± 1·22	27·8 ± 0·9	NS
Body fat (%)	30·4 ± 0·01	33·1 ± 0·01	NS

Table 2. Clinical characteristics and treatment of 10 GH-deficient patients treated with GH

Patient no.	Sex	Aetiology	Treatment
1	M	Pituitary adenoma	LT4, SS, HC
2	M	Haemangioma	LT4, SS, HC
3	M	Post-traumatic	LT4, SS, HC, D
4	F	Sheehan	LT4, HC
5	F	Pituitary adenoma	LT4, SS, D
6	F	Pituitary adenoma	LT4, SS, HC
7	F	Pituitary adenoma	LT4, SS, HC
8	F	Sheehan	LT4, SS, HC
9	F	Sheehan	LT4, SS, HC
10	F	Sheehan	LT4, SS, HC

M, male; F, female; LT4, L-thyroxine; SS, sex steroids; HC, hydrocortisone; D, desmopressin.

recombinant. Clinical data concerning these patients are given in Table 2. All patients had adult-onset GHD and were equilibrated concerning the different pituitary insufficiencies before GH treatment. A physical activity questionnaire was completed and subjects were asked to maintain their exercise habits.

Initial instructions

Subjects were asked to fast overnight before testing and take substitutive hormonal treatment as usual.

Anthropometry

Weight, height, waist and hip measurements were obtained and body mass index (BMI) was calculated as weight in kilograms divided by height in metres squared (kg/m^2). Body composition as fat mass and FFM was assessed with a multifrequency bioelectrical impedance instrument (Dietosystem Human IM Scan) using the following frequencies: 1, 5, 10, 50 and 100 kHz. Analysis was performed with the software Master 1·0.

Borg scale

The rating of perceived exertion (RPE) was assessed with the Borg scale, quoting RPE values between 0 and 10.[18]

Treatment

Administration of GH was initiated at a dose of 0·15–0·2 mg/day by nocturnal subcutaneous injection and increased progressively to 0·2–0·6 mg/day according to clinical manifestation, and targeted to normalize IGF-I values according to sex and age. The dose of GH treatment at 12 months was optimized and higher than at 6 months.

Exercise testing

The subjects performed an exercise test on an electromagnetically braked cycle ergometer (Ergoline Bosh 500) connected to a breath-by-breath device (ZAN 600) for gas exchange measurements (VO_2 and VCO_2).

To individualize the increment of exercise testing,[19,20] the workload of each step was calculated from the theoretical maximal aerobic power (Wmax th), that is power corresponding to the theoretical VO_2max. These theoretical values were calculated before the exercise test as described previously.[19,20] Consequently, the subjects underwent a test with the same relative incremental workload and were compared at the same percentage of their Wmax th.

The test consisted of five 6-min steady-state workloads corresponding to 20%, 30%, 40%, 50% and 60% of Wmax th and ended by incremental steps of 1 min to maximal power workload. Heart rate was monitored continuously throughout the test. Ventilatory parameters (VO_2, VCO_2) were recorded during the last 3 min of each workload. Pedal frequency was maintained at 60–70 rev/min throughout the test.

Fat and CHO oxidation

Whole-body substrate oxidation was calculated from the measurement of the respiratory exchange ratio (RER = VCO_2/VO_2 in expired gases). VO_2 and VCO_2 were determined as the means of measurements during the last 3 min of each workload, according to MacRae *et al.*[14,15,21,22]

The percentages of CHO and fat oxidation were calculated by using the following equations:[23]

$$\%\ \text{CHO} = [(\text{RER} - 0{\cdot}71)/0{\cdot}29] \times 100$$

$$\%\ \text{Fat} = [(1 - \text{RER})/0{\cdot}29] \times 100$$

Fat and CHO oxidation rates were calculated from the gas exchange measurements according to the nonprotein respiratory quotient technique:[24]

$$\text{CHO(mg/min)} = 4{\cdot}585\ VCO_2 - 3{\cdot}2255\ VO_2$$

$$\text{Fat(mg/min)} = -1{\cdot}7012\ VCO_2 + 1{\cdot}6946\ VO_2$$

with gas volume expressed in ml/min. CHO and fat oxidation rate were normalized by FFM. This calculation provides a determination of the proportion of CHO and fat, and thus provides a simplified evaluation of the balance of substrates oxidized by the body assuming that the part taken by protein is negligible during a 30 min of exercise.

After smoothing the curves, we calculated two parameters representative of the balance between fat and CHO utilization induced by increasing exercise intensity: the maximal fat oxidation point (Lipox max)[22] and the crossover point. Lipox max is the point

where the increase in fat oxidation induced by increasing workload reaches a maximum, which will then be followed by a decrease as CHO becomes the predominant fuel. It is calculated as described in our previous papers on the balance of substrates in children[15] and adults.[22] Crossover point is defined as the power at which energy from CHO-derived fuels predominates over energy from lipids.[22]

Laboratory measurement

Serum IGF-I was assessed with a Mediagnost radioimmunoassay (Germany). The sensitivity was 0·1 ng/ml with a between-assay coefficient of variation of 7·4%.

Data analysis

Comparison of different parameters of body composition and metabolic parameters during exercise were performed with the Student *t*-test for unpaired data. Significance of differences with the treatment on 6 and 12 months were determined by using repeated-measures analysis of variance (ANOVA). Post-hoc comparisons were made using Sheffe's test for significant difference. For all statistical analyses, values were expressed as mean (± SEM) and significance was accepted at $P < 0{\cdot}05$.

Results

Cross-sectional study

Anthropometric characteristics. Patients appeared to be well matched for age, sex and BMI (Table 1). However, the waist-to-hip ratio was different between GHD and control groups (0·87 vs. 0·81, respectively, P = 0·002), suggesting that the mass of abdominal adipose tissue was higher in the GHD group.

Substrate oxidation during exercise. Figure 1 shows the crossover point and Lipox max in the GHD and control groups. The crossover point of substrate oxidation was significantly lower in the GHD group than in the control group (52 ± 5·5% *vs.* 72·6 ± 6·6% of Wmax th) ($P < 0{\cdot}03$). Lipox max shows a tendency to decrease in the GHD group but this decrease was not significant ($P < 0{\cdot}06$). The lipid oxidation rate at the level of the Lipox max was significantly decreased in the GHD group compared with the control group ($P < 0{\cdot}001$) (Fig. 2).

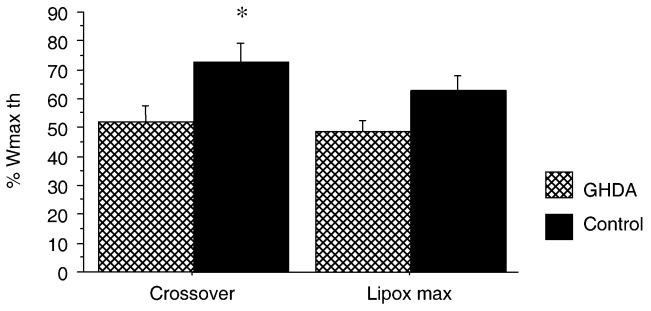

Fig. 1 Comparison of substrate oxidation parameter (in percentage Wmax th) in the GHD group and controls. *$P < 0{\cdot}03$.

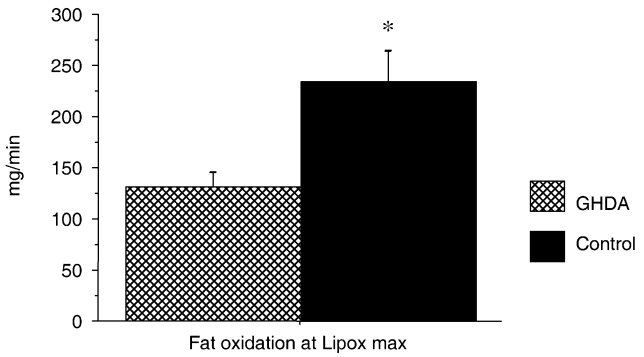

Fig. 2 Comparison of fat oxidation at Lipox max (mg/min) in the GHD group and controls. *$P < 0{\cdot}001$.

Follow-up study

Anthropometric characteristics. For the same BMI, FFM (in kg) increased significantly after this treatment (P = 0·01). The post hoc Sheffe test showed a significant difference between measurements performed at 0 and 6 months (P = 0·04), and between those performed at 0 and 12 months (P = 0·04) (Fig. 3).

Hormonal parameters. Plasmatic IGF-I concentration was increased significantly after GH treatment (61·2, 157·4 and 187·4 ng/ml at T0, T6 and T12, respectively) ($P = 0{\cdot}009$).

Substrate oxidation. The maximal power increased significantly after treatment (102 ± 16, 121·6 ± 18 and 137 ± 25 W at T0, T6 and T12, respectively) ($P < 0{\cdot}01$). Figure 4 shows that the crossover point, expressed in percentage Wmax th, was increased significantly with treatment ($P < 0{\cdot}02$). The same result was found when crossover point and Lipox max were expressed in Watts (35·1 ± 7·5 W at T0 *vs.* 47·9 ± 4·1 W at T12, $P < 0{\cdot}03$, 40·3 ± 5·4 W at T0 *vs.* 49·6 ± 7·1 W at T12, $P < 0{\cdot}05$). Additionally, fat oxidation at the level of the Lipox max was significantly increased with treatment

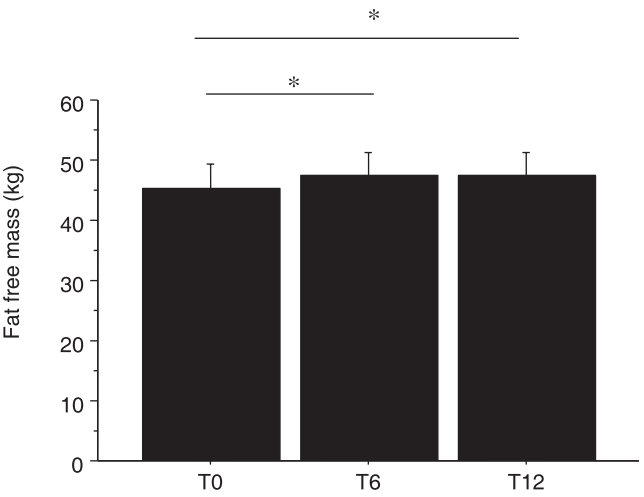

Fig. 3 Evolution of fat free mass (kg) after GH treatment at 6 and 12 months (T6 and T12) in comparison to T0. *$P < 0{\cdot}04$.

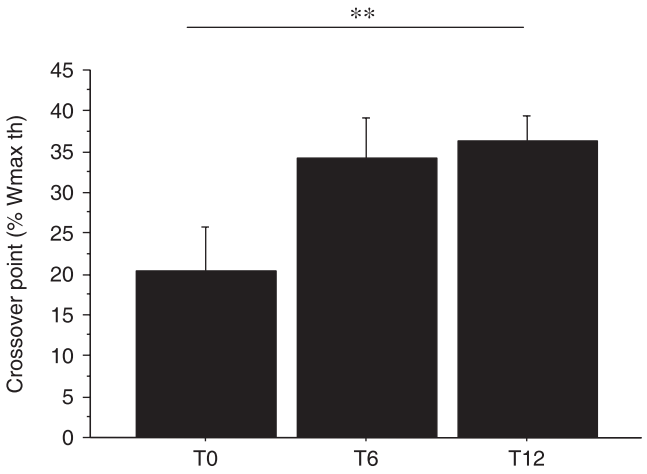

Fig. 4 Effect of 6 and 12 months (T6 and T12) of GH treatment on crossover point expressed as percentage of Wmax th. $*P < 0{\cdot}02$.

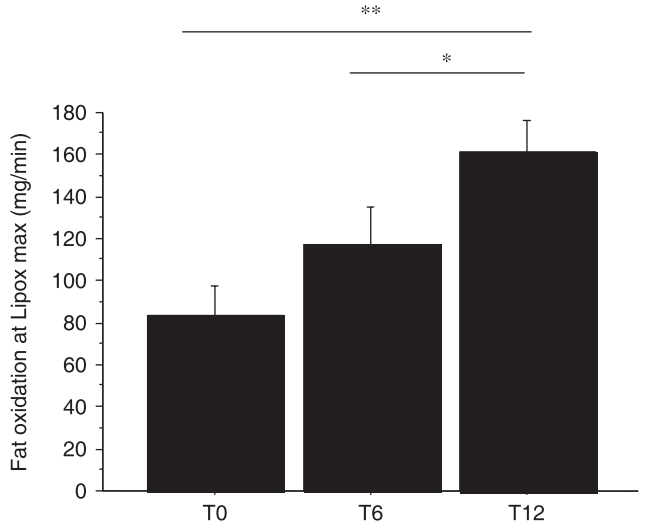

Fig. 5 Effect of 6 and 12 months (T6 and T12) of GH treatment on fat oxidation at Lipox max expressed in mg/min. $*P < 0{\cdot}04$; $**P < 0{\cdot}001$.

($80 \pm 11{\cdot}9$, $104{\cdot}8 \pm 16{\cdot}4$ and $146{\cdot}5 \pm 16{\cdot}7$ mg/min at T0, T6 and T12, respectively) ($P < 0{\cdot}001$) (Fig. 5). The RPE assessed with the Borg scale decreased along treatment time when it was measured at 30% Wmax th ($P < 0{\cdot}01$) (Fig. 6).

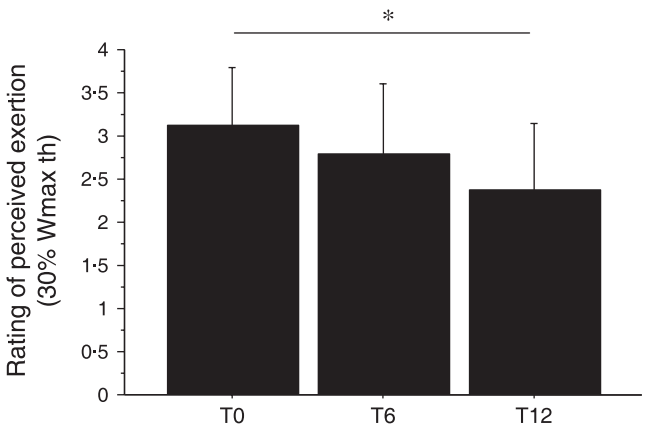

Fig. 6 Effect of 6 and 12 months (T6 and T12) of GH treatment on the rating of perceived exertion (RPE) on the Borg scale quoting exercise from 0 to 10 calculated at 30% Wmax th. $*P < 0{\cdot}01$.

Discussion

The main result of this study is that GH-deficient patients, compared to controls matched for age, sex and BMI, exhibit a highly significant change in the balance of substrate oxidation during exercise, towards a decrease in fat oxidation, and a shift towards lower intensities of the powers at which the crossover and the maximal fat oxidation rate occur. Therefore, there is impairment in the ability to oxidize fat during exercise in GH-deficient patients. However, GH treatment partially reverses this defect and restores fat oxidation during exercise.

Specific exercise tests designed for measuring the balance of substrates at exercise in either athletes[25] or patients[22] have only recently been introduced, because it has been difficult to convince physicians and scientists that calorimetric equations could be used accurately during graded exercise tests. Contrasting with long duration (> 20 min) steady-state workloads, physiologists were concerned with the stability of gas exchanges during steps of exercise shorter than 10 min. Based on our previous studies on calorimetry during long duration steady-state workloads,[16] we developed a test[22] consisting of five 6-min submaximal steps, in which we assumed that a steady state for gas exchanges was obtained during the last 2 minutes. In fact, there is now a large body of literature to support the validity of such protocols of exercise calorimetry.[26] The theoretical concern was that, when exercise is performed above the lactate threshold, there is an extra CO_2 production that can be assumed to interfere with the calculations.[21] However, below 75% of the VO_2max, this increase in CO_2 has no measurable effect on calorimetric calculations[27] and thus these calculation closely predict the actual oxidation rates that could be measured with much more sophisticated and expensive approaches such as stable isotope labelling.[27] Clearly, even at high intensity exercise, respiratory gases are mostly the reflection of the balance of substrate oxidation. In addition, despite the theoretical uncertainty about the stability of gas exchanges during such short bouts, protocols of graded exercise calorimetry with only 3-min duration steps have been carefully validated and used successfully,[25,28,29] further supporting the accuracy of our protocols based on 6-min steps. In general, we consider that graded exercise tests for exercise calorimetry can currently be considered as validated. In the case of GHD and GH treatment, it could be argued that important changes in protein metabolism may be expected that cannot be taken into account with this method, which assumes that energy expenditure increases dramatically during exercise and the role taken by protein oxidation can be neglected. In fact, even if there were a twofold increase in protein oxidation, it is easy to calculate that this would have only a mirror effect on the balance between CHO and lipid, given the high values of oxidation rates that are reached. In addition, it has been recently demonstrated that acute GH administration does not significantly impact protein metabolism during exercise.[30] Thus, although a specific study on protein oxidation during exercise in

patients with GHD and during GH treatment remains to be done, we consider that our calculations of exercise calorimetry remain valid under the conditions of this study.

With this technique, we find significant changes in the ability to oxidize lipids in GH-deficient patients when they are treated with GH. The balance of substrates, which was shifted towards a predominance of CHO compared to the control group, changes after GH treatment, towards an increase in lipid oxidation during exercise. Because of the major effects of the GH–IGF-I axis on body composition, it is logical to assume that changes in FFM and fat mass explain most of these metabolic alterations. However, these relationships between body composition and the balance of substrates at exercise do not appear clearly in this study (despite a highly significant increase in FFM after GH treatment). This is probably because in one patient treated with GH there was a paradoxical increase in fat mass due to modifications in his eating behaviour. In two preceding studies in obese patients submitted to regular training,[14,15] we observed that the changes in the balance of substrates at exercise appeared earlier than the changes in body composition. Whether this is also the case in GH-deficient patients remains to be studied. Obviously, most of the effect of GH on body composition is due to its metabolic actions at rest, but the alterations of exercise fuel metabolism may be a sensitive and early marker of overall GH effects on the balance of substrates. Further studies are needed to clarify this issue.

These results are thus likely to indicate that GH plays an important role in the regulation of fuel selection at exercise. As discussed above, GH-induced modifications of body composition are quite moderate and are not likely to explain an important part of this effect. It seems likely therefore that the metabolic actions of GH (on its own or mediated by IGF-I) are the major explanation for this effect. It has been shown that acute administration of GH leads to a subsequent increase in plasma glycerol and nonesterified fatty acids (NEFAs) at rest, indicating increased lipolysis,[31,32] and such a mechanism could explain to some extent this increase in fat oxidation. However, very limited information is available on metabolic effects during exercise of prolonged GH administration. As GH induces a rise in IGF-I, the effect of IGF-I on the metabolic changes observed here should be discussed. In a preceding cross-sectional study in professional soccer players, we found that serum levels[33] of IGF-I and IGFBP3 correlated with the ability to oxidize lipid during exercise,[34] suggesting that an enhanced secretory ability of the somatotrophic axis improves lipid oxidation at exercise. However, recent studies show that IGF-I at physiological doses does not increase lipid oxidation but rather decreases it,[35] an effect that appears to be blunted in diabetes and is probably also impaired during exercise due to IGF-I trapping by its binding proteins. A stimulatory effect of IGF-I on lipid oxidation has only been described at supraphysiological concentrations and is thus very unlikely to explain our findings. According to the bulk of the literature on this subject, it seems logical to interpret the changes in lipid oxidation as mostly resulting from the lipolytic action of GH. Recent studies investigating the metabolic effects of an acute exposure to GH during muscular activity mostly report regulation of lipid metabolism with little or no effect on proteins and glucose.[30] Therefore, alterations of lipid oxidation under GH treatment may be due to a direct GH effect rather than an IGF-I-mediated effect. These findings are consistent with the hypothesis that a lack of GH reduces the ability to oxidize lipids during exercise and that GH treatment restores this muscular metabolic property.

In conclusion, this study finds evidence of impairment in the ability to oxidize lipids at exercise in GHD, and its reversal after long-term GH treatment. Therefore, besides its increasing effects on FFM, GH may regulate the ability of muscle to oxidize lipids at exercise, so that GH treatment restores this metabolic process, which is impaired in GH-deficient adults.

Acknowledgements

This study was supported by a research grant from Pfizer Inc. (France). We thank Magali Bonnet, Marie-Chantal Granat and Katie Mezes for technical assistance.

References

1 Jorgensen, J.O., Pedersen, S.A., Thuesen, L., Jorgensen, J., Ingemann-Hansen, T., Skakkebaek, N.E. & Christiansen, J.S. (1989) Beneficial effects of growth hormone treatment in GH-deficient adults. *Lancet*, **1**, 1221–1225.

2 Binnerts, A., Deurenberg, P., Swart, G.R., Wilson, J.H. & Lamberts, S.W. (1992) Body composition in growth hormone-deficient adults. *American Journal of Clinical Nutrition*, **55**, 918–923.

3 Jorgensen, J.O., Moller, J., Alberti, K.G., Schmitz, O., Christiansen, J.S., Orskov, H. & Moller, N. (1993) Marked effects of sustained low growth hormone (GH) levels on day-to-day fuel metabolism: studies in GH-deficient patients and healthy untreated subjects. *Journal of Clinical Endocrinology and Metabolism*, **77**, 1589–1596.

4 Rosen, T., Eden, S., Larson, G., Wilhelmsen, L. & Bengtsson, B.A. (1993) Cardiovascular risk factors in adult patients with growth hormone deficiency. *Acta Endocrinologica (Copenhagen)*, **129**, 195–200.

5 Cuneo, R.C., Salomon, F., Wiles, C.M. & Sonksen, P.H. (1990) Skeletal muscle performance in adults with growth hormone deficiency. *Hormone Research*, **33**, 55–60.

6 Salomon, F., Cuneo, R.C., Hesp, R. & Sonksen, P.H. (1989) The effects of treatment with recombinant human growth hormone on body composition and metabolism in adults with growth hormone deficiency. *New England Journal of Medicine*, **321**, 1797–1803.

7 Amato, G., Carella, C., Fazio, S., La Montagna, G., Cittadini, A., Sabatini, D., Marciano-Mone, C., Sacca, L. & Bellastella, A. (1993) Body composition, bone metabolism, and heart structure and function in growth hormone (GH)-deficient adults before and after GH replacement therapy at low doses. *Journal of Clinical Endocrinology and Metabolism*, **77**, 1671–1676.

8 De Boer, H., Blok, G.J., Voerman, H.J., De Vries, P.M. & van der Veen, E.A. (1992) Body composition in adult growth hormone-deficient men, assessed by anthropometry and bioimpedance analysis. *Journal of Clinical Endocrinology and Metabolism*, **75**, 833–837.

9 Czernichow, P. (1993) Growth hormone administration and carbohydrate metabolism. *Hormone Research*, **39**, 102–103.

10 Fedou, C., Brun, J.F., Raynaud, E., Boyer, P., Rodier, M., De Boisvilliers, F., Mouillon, N. & Orsetti, A. (1996) Insulin sensitivity and glucose effectiveness measured with the minimal model in adults with GH deficiency. *Endocrinology and Metabolism*, **3**, 99–104.

11 De Boer, H., Blok, G.J. & Van der Veen, E.A. (1995) Clinical aspects of growth hormone deficiency in adults. *Endocrine Reviews*, **16**, 63–86.

12 Carroll, P.V., Christ, E.R., Bengtsson, B.A., Carlsson, L., Christiansen, J.S., Clemmons, D., Hintz, R., Ho, K., Laron, Z., Sizonenko, P., Sonksen, P.H., Tanaka, T. & Thorne, M. (1998) Growth hormone deficiency in adulthood and the effects of growth hormone replacement: a review. Growth Hormone Research Society Scientific Committee. *Journal of Clinical Endocrinology and Metabolism*, **83**, 382–395.

13 Bengtsson, B.A., Eden, S., Lonn, L., Kvist, H., Stokland, A., Lindstedt, G., Bosaeus, I., Tolli, J., Sjostrom, L. & Isaksson, O.G. (1993) Treatment of adults with growth hormone (GH) deficiency with recombinant human GH. *Journal of Clinical Endocrinology and Metabolism*, **76**, 309–317.

14 Dumortier, M., Brandou, F., Perez-Martin, A., Fedou, C., Mercier, J. & Brun, J.F. (2003) Low intensity endurance exercise targeted for lipid oxidation improves body composition and insulin sensitivity in patients with the metabolic syndrome. *Diabetes and Metabolism*, **29**, 509–518.

15 Brandou, F., Dumortier, M., Garandeau, P., Mercier, J. & Brun, J.F. (2003) Effects of a two-month rehabilitation program on substrate utilization during exercise in obese adolescents. *Diabetes and Metabolism*, **29**, 20–27.

16 Manetta, J., Brun, J.F., Perez-Martin, A., Callis, A., Prefaut, C. & Mercier, J. (2002) Fuel oxidation during exercise in middle-aged men: role of training and glucose disposal. *Medicine and Science in Sports and Exercise*, **34**, 423–429.

17 Growth Hormone Research Society (1998) Consensus guidelines for the diagnosis and treatment of adults with growth hormone deficiency: summary statement of the Growth Hormone Research Society Workshop on Adult Growth Hormone Deficiency. *Journal of Clinical Endocrinology and Metabolism*, **83**, 379–381.

18 Borg, G.A. (1982) Psychophysical bases of perceived exertion. *Medicine and Science in Sports and Exercise*, **14**, 377–381.

19 Wasserman, K., Hansen, J. & Whipp, B. (1986) *Principles of Exercise Testing and Interpretation.* Lea & Febiger, Philadelphia, 50–80.

20 Serres, I., Gautier, V., Varray, A. & Prefaut, C. (1998) Impaired skeletal muscle endurance related to physical inactivity and altered lung function in COPD patients. *Chest*, **113**, 900–905.

21 MacRae, H.S., Noakes, T.D. & Dennis, S.C. (1995) Role of decreased carbohydrate oxidation on slower rises in ventilation with increasing exercise intensity after training. *European Journal of Applied Physiology and Occupational Physiology*, **71**, 523–529.

22 Perez-Martin, A., Dumortier, M., Raynaud, E., Brun, J.F., Fedou, C., Bringer, J. & Mercier, J. (2001) Balance of substrate oxidation during submaximal exercise in lean and obese people. *Diabetes and Metabolism*, **27**, 466–474.

23 MacGilvery, R.W. & Goldstein, G.W. (1983) *Biochemistry: A Functional Approach*. Saunders, Philadelphia, 810–976.

24 Peronnet, F. & Massicotte, D. (1991) Table of nonprotein respiratory quotient: an update. *Canadian Journal of Sport Sciences*, **16**, 23–29.

25 Achten, J. & Jeukendrup, A.E. (2003) Maximal fat oxidation during exercise in trained men. *International Journal of Sports Medicine*, **24**, 603–608.

26 Jeukendrup, A.E. & Wallis, G.A. (2005) Measurement of substrate oxidation during exercise by means of gas exchange measurements. *International Journal of Sports Medicine*, **26**, S28–S37.

27 Romijn, J.A., Coyle, E.F., Sidossis, L.S., Gastaldelli, A., Horowitz, J.F., Endert, E. & Wolfe, R.R. (1993) Regulation of endogenous fat and carbohydrate metabolism in relation to exercise intensity and duration. *American Journal of Physiology*, **265**, E380–E391.

28 Achten, J., Gleeson, M. & Jeukendrup, A.E. (2002) Determination of the exercise intensity that elicits maximal fat oxidation. *Medicine and Science in Sports and Exercise*, **34**, 92–97.

29 Venables, M.C., Achten, J. & Jeukendrup, A.E. (2005) Determinants of fat oxidation during exercise in healthy men and women: a cross-sectional study. *Journal of Applied Physiology*, **98**, 160–167.

30 Kanaley, J.A., Dall, R., Moller, N., Nielsen, S.C., Christiansen, J.S., Jensen, M.D. & Jorgensen, J.O. (2004) Acute exposure to GH during exercise stimulates the turnover of free fatty acids in GH-deficient men. *Journal of Applied Physiology*, **96**, 747–753.

31 Keller, U., Schnell, H., Girard, J. & Stauffacher, W. (1984) Effect of physiological elevation of plasma growth hormone levels on ketone body kinetics and lipolysis in normal and acutely insulin-deficient man. *Diabetologia*, **26**, 103–108.

32 Lange, K.H., Larsson, B., Flyvbjerg, A., Dall, R., Bennekou, M., Rasmussen, M.H., Orskov, H. & Kjaer, M. (2002) Acute growth hormone administration causes exaggerated increases in plasma lactate and glycerol during moderate to high intensity bicycling in trained young men. *Journal of Clinical Endocrinology and Metabolism*, **87**, 4966–4975.

33 Mauras, N. & Haymond, M.W. (2005) Are the metabolic effects of GH and IGF-I separable? *Growth Hormone and IGF Research*, **15**, 19–27.

34 Brun, J.F., Fedou, C. & Chaze, D. (1999) Relationships between the balance of lipids and carbohydrates oxidized at various levels of exercise and the GH-IGF-1-IGFBP axis in trained athletes. XVIIème Congrès de la Société Française d'endocrinologie. *Annales d'Endocrinologie*, **60**, 366.

35 Pratipanawatr, T., Pratipanawatr, W., Rosen, C., Berria, R., Bajaj, M., Cusi, K. *et al.* (2002) Effect of IGF-I on FFA and glucose metabolism in control and type 2 diabetic subjects. *American Journal of Physiology. Endocrinology and Metabolism*, **282**, E1360–E1368.

